Machine based registration of light and electron microscopy images of serial ultrathin sections to reconstruct specimens in three dimensions

Christian Wojek[1], Jochen Fuchs[1] and Christian Dietrich[2]

1. Carl Zeiss AG, Carl-Zeiss-Strasse 22, 73447 Oberkochen, Germany
2. Carl Zeiss AG, Carl Zeiss Promenade 10, 07745 Jena, Germany

Email contact of corresponding author: c.dietrich@zeiss.de
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Light and electron microscopy are highly successful technologies when used independently, and are increasingly used together by researchers in the field of Correlative Microscopy. This combines the strength of different microscope technologies to gain new insights into the functionality and the associated ultra-structure of biological specimens. One popular approach to image three-dimensional objects is to slice them into ordered arrays of ultrathin, resin- embedded sections and reconstruct the volume from the recorded images [1].

In our approach, the user first outlines one section and an automated image analysis discovers the other sections on the substrate. Knowledge of the precise location and orientation of single slices then allows the user to define volumes of interest (VOI) even when sections are slightly disrupted or bent. These regions of interest are imaged with an accuracy of a few microns using both types of microscope [2], and the images are then registered slice-by-slice. To achieve an automated and robust registration of 2D images obtained by dissimilar modalities (see Fig. 1A), we record an additional contrast that is perfectly aligned to one of the modalities and that allows precise alignment to the second modality. After the identification of correlative features [3], the discriminative patterns are described using a variety of different feature descriptors [4, 5]. Comparing the high-dimensional features (Fig. 2) allows correspondences to be established and the transformation parameters to be optimized using robust Monte Carlo hypothesize-and-test methods [6]. Finally, the registered 2D images of individual slices are aligned in a similar manner by a slice registration procedure to reconstruct the full three-dimensional volume.

Our workflow is designed to limit user interaction to as few initialization and correction steps as possible, thus enabling a nearly fully automated alignment of LM and EM data. In our experiments we typically achieve an alignment accuracy in the range of the resolution of the LM images. [7]

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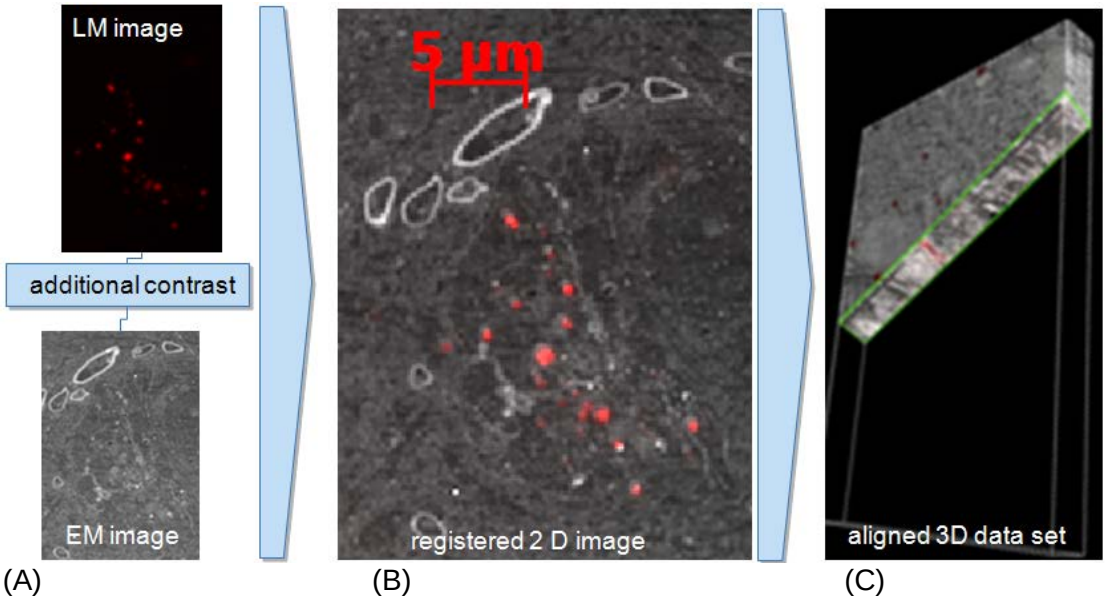

Figure 1 *Region of interest within one ultrathin brain section imaged with different microscope contrasts (A). Overlay of a fluorescence LM image and an SEM image (B). Reconstructed 3D volume (C).*

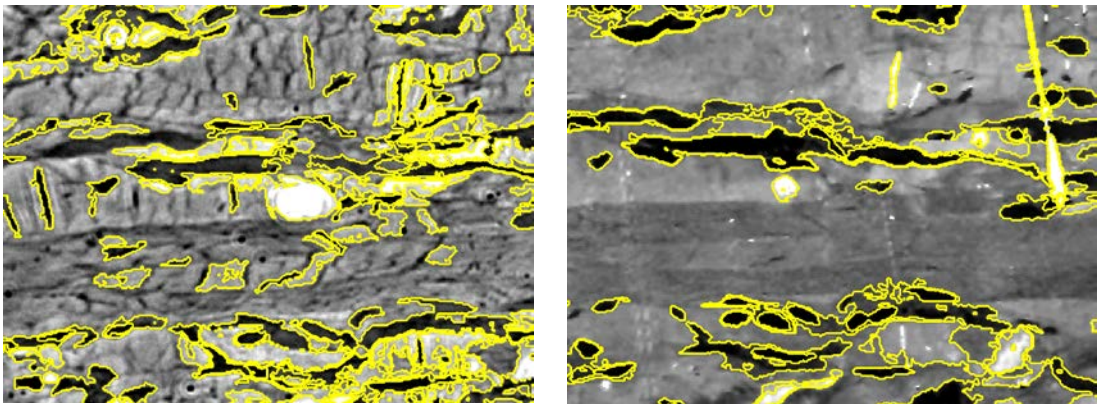

Figure 2 *Determination of characteristic features in the same region of interest of a skin section. A phase contrast image is shown in (A). (B) depicts an electron microscope image detected with a secondary electron detector. For both contrasts, identified features are outlined with yellow overlay.*